CHARACTERISTICS OF THE CHEMICAL NATURE OF CERTAIN BACTERIAL ALLERGENS

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CHARACTERISTICS OF THE CHEMICAL NATURE OF CERTAIN BACTERIAL ALLERGENS

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Bacterial allergy has an important role in the pathogenesis of many infectious diseases. Determination of heightened sensitivity of the organism to various microbe species is hampered by the absence of unified methods of producing qualitatively heterogenic preparations which are applicable for diagnosis of bacterial allergy. In connection with this the question of the biochemical decoding of allergens, identifying localization of the active principle in a certain chemical substance, and the possibility of isolating it in pure form represent substantial theoretical and practical interest,- the more so, since insufficient work has been done in this area.

We carried out investigations of the chemical composition of allergens from Streptococcus hemolyticus obtained by two methods - the Ando-Verzhikovskiy* (1936) and Val'* and that of Drash* and Kaye* (1964).

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*Translator's Note - Names marked with (*) are probable non-Soviet whose spelling has not been verified.

Strains of group A Str. hemolyticus were selected with consideration of recommendations in MRTU-42 for the production of this form of allergen. The biological activity of the preparations was determined by means of skin-allergic titration on all CRL diseases. The age group 18-25 years made up the maximum number of patients and the predominate disease was chronic tonsillitis.

Protein in the allergen preparations was determined by the Kjeldahl and Folin-Lowry methods and by UV absorption at 280 mu on an SF-4 spectrophotometer, while reducing substances and glucides were determined by the Molish* and Trevelyan* methods. Nucleic acids and their derivatives were determined by the Dische, Meybaum*, Spirin, Inglender* and Epshteyn*, and Tsanev and Markov methods. Chromatography was carried out on columns with DEAE and OA [oxygen-absorption] sephadexes; all spectrophotometric determinations were made on the SF-4A. Studies were made of the chemical composition of six series of allergen from Str. hemolyticus obtained by the Ando-Verzhikovskiy method and nine series obtained by the method of Val', Drash, and Kaye (in our own modification).

The preparations obtained by the two methods turned out to be heterogenic and, in addition to protein, contained derivatives of nucleic acids and glucides (Table 1).

Table 1. Chemical composition of allergens of Str. hemolyticus obtained by various methods (averaged data).

Chemical components of the allergens	Preparation method			
	Ando-Verzhikovskiy		Val' et al.	
	ν/ml	%	ν/ml	%
Protein	291,6	55	343,1	50
Glucides	32,3	6,1	60,5	8,8
RNA (derivatives)	159,2	30,1	203,1	29,6
DNA (derivatives)	44,2	8,8	79,5	11,6

Up to now it has been customary to regard the thermostable fraction of streptococcal toxin obtained by the Ando-Verzhikovskiy method as a nucleoprotein, as proposed by Ando (1930).

Our preliminary investigations showed that the thermostable fraction does not contain any polymeric nucleic acids, but has only structural fragments of RNA and DNA molecules with various degrees of polymerism, which are not able to form a stable complex with protein - i.e., nucleoprotein. These data do not support the conclusions of a number of authors who detected RNA in the thermostable fraction and did not find DNA. It is possible that the reason for such divergence is the fact that in all cases a test with orsin on pentose was applied; this made it possible to differentiate the polymeric nucleic acid from its derivatives found in this carbohydrate.

The protein in the allergen composition also cannot be considered homogeneous, since after chromatography on a column with DEAE sephadex (a weak anionite) we obtained five peaks, four of which were protein and one nucleotide. We were unable to achieve ideal purification of the protein material; the nucleotide peak partially overlapped the first three protein peaks, although its maximum occupied a separate position. However, even these prelimiary data indicate the heterogeneity of the protein fraction of the allergen from Str. hemolyticus.

The chemical composition of the alcohol-acid extract obtained per Val', Drash, and Kaye turned cut to be very similar to the thermostable fraction (considering the quantitative relationship of the individual components). On the other hand, certain qualitative differences were noted: in the alcohol-acid extract about 63% of the RNA was in the polymer state, with DNA also remaining polymeric by 8-12%.

We made an effort to fractionate the obtained alcohol-acid extracts. French authors used chromatography on KM-cellulose for this purpose. Column dimensions were 2x25 cm, with an elution rate of about 15 ml/h and fraction volume of 4 mz each. The cationite was balanced with an acetate-sodium buffer, 0.01 M, pH 4.2; with stepwise elution with the same buffer and then 0.01N HCl and 0.02N HCl. Three protein peaks quite clearly separated from one another were obtained; in this case nucleotide material was distributed over all protein peaks.

For removal of nucleotide material the third protein peak was subjected to rechromatography under the same conditions. In the end it was possible to free the most massive protein peak from contamination by nucleotide material.

We found it advisable to defractionate the alcohol-acid extract on an anionite, which is more suitable for separation of basic proteins. For this purpose we prepared a column of DEAE sephadex A-50, with column dimensions of 25×2.5 cm; elution was with a *tris*-HCl buffer, pH 7.2, in a linear gradient of sodium chloride 0.05-0.5 M, fractions of 5 ml each. Flow rate was 15 ml/h. Chromatography was carried out at a temperature of +6-8°C.

Once again three protein fractions were obtained, two of which were contaminated with nucleotide material. Similar results were obtained when the *tris*-HCl buffer was replaced with a phosphate buffer with pH 6.8.

We regard the obtained data as the first step in preparing highly purified and specific bacterial allergen preparations.